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   1 TTTCCTCACTGACTATAAAAGAATAGAGAAGGAAGGGCTTCAGTGACCGGCTGCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC

  91 ATGGCTATGATGGAGGTCCAGGGGGGACCCAGCCTGGGACAGACCTGCGTGCTGATCGTGATCTTCACAGTGCTCCTGCAGTCTCTCTGT
   1 MetAlaMetMetGluValGlnGlyGlyProSerLeuGlyGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys

     GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGGCATTGCTTGTTTCTTAAAAGAA
     ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPheLeuLysGlu

 271 GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG
  61 AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys

 361 ATGATTTTGAGAACCTCTGAGGAAACCATTTCTACAGTTCAAGAAAAGCAACAAAATATTTCTCCCCTAGTGAGAGAAAGAGGTCCNCAG
  91 MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnGlnAsnIleSerProLeuValArgGluArgGlyProGln

 451 AGAGTAGCAGCTCACATAACTGGGACCAGAGGAAGAAGCAACACATTGTCTTCTCCAAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAA
 121 ArgValAlaAlaHisIleThrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys

 541 ATAAACTCCTGGGAATCATCAAGGAGTGGGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGTGAACTGGTCATCCATGAAAAAGGG
 151 IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly

 631 TTTTACTACATCTATTCCCAAACATACTTTCGATTTCAGGAGGAAATAAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATATT
 181 PheTyrTyrIleTyrSerGlnThrTyrPheArgPheGlnGluGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle

 721 TACAAATACACAAGTTATCCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGACTCTAT
 211 TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr

 811 TCCATCTATCAAGGGGGAATATTTGAGCTTAAGGAAAATGACAGAATTTTTGTTTCTGTAACAAATGAGCACTTGATAGACATGGACCAT
 241 SerIleTyrGlnGlyGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis

 901 GAAGCCAGTTTTTTCGGGGCCTTTTTAGTTGGCTAACTGACCTGGAAAGAAAAAGCAATAACCTCAAAGTGACTATTCAGTTTTCAGGAT
 271 GluAlaSerPhePheGlyAlaPheLeuValGlyStp

 991 GATACACTATGAAGATGTTTCAAAAAATCTGACCAAAACAAACAAACAGAAA
```

FIG. 1

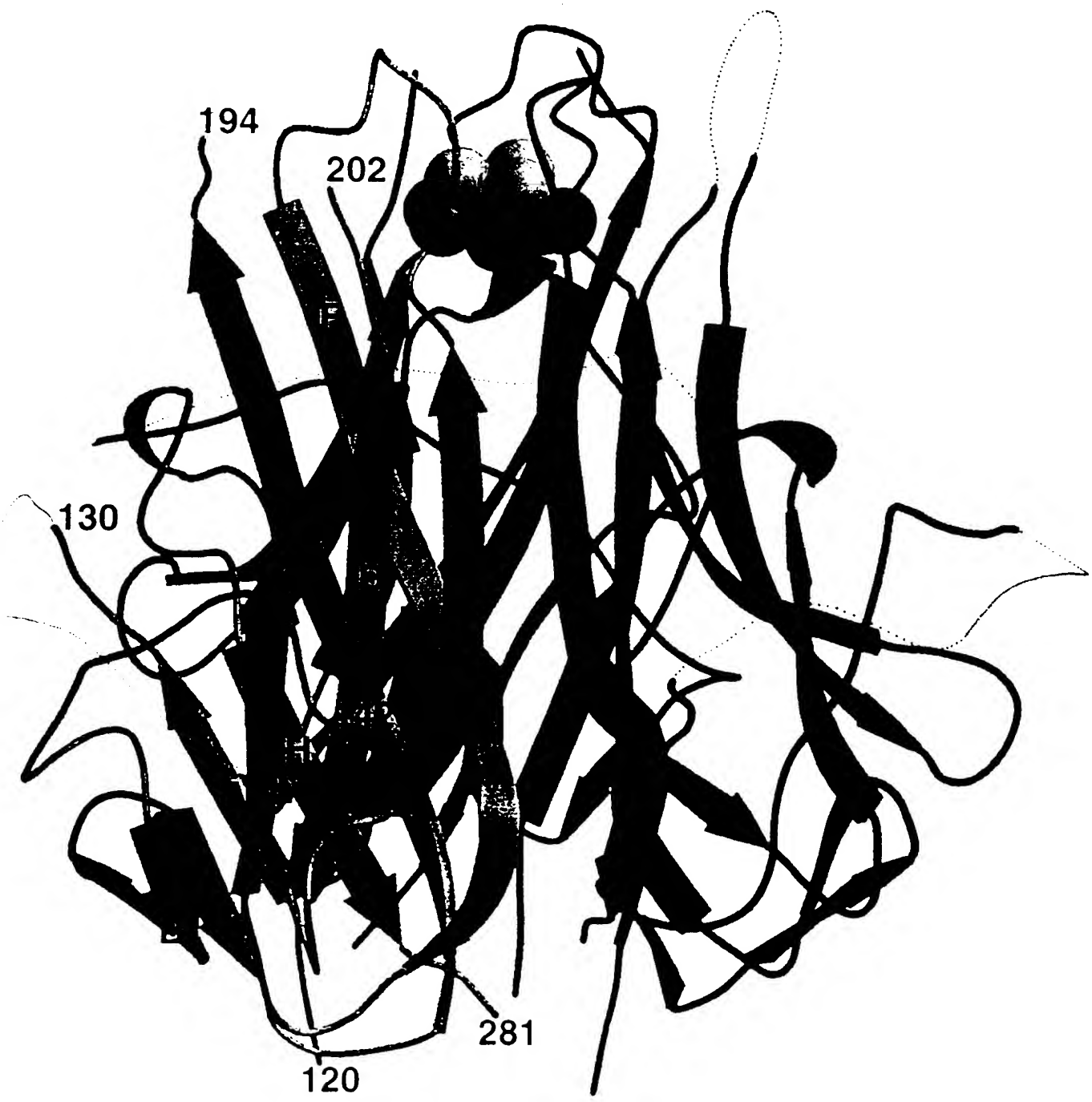


Figure 2A

Figure 2B

Crystallographic Data

	Apc-2L(114-281)	Apo-2L(91-281)D218A	Apc-2L(91-281) D218A
Crystal			
Space Group	$P6_3$	R32	$R\bar{3}2$
Unit Cell (Å)	a=72.5 c=140	a=66.4 c=197.6	a=66.4 c=197.7
Resolution (Å)	3.9	1.9	1.3
Coverage (%)	94 (96)	93 (99)	100 (100)
$\langle I/\sigma(I)\rangle$	5.9	10.1	12.4
# unique (hkl)	3589	12680	41840
redundancy	4.9	4.3	12.1
R_{symm} (%)	15.4 (34)	6.2 (27)	6.4 (34)
# protomers in ASU	2	1	1
Refinement			
R_{cryst} (%)	33.8	20	
R_{free} (%)	27.6	32	
rmsd bonds (Å)	0.009	0.015	0.007
rmsd angles (°)	1.79	2.0	1.41
average B-values	--	14	14
# water molecules	0	170	

$R_{symm} = \Sigma_h\Sigma_i(I_{hi}-\langle I_h\rangle)/\Sigma_h I$ where I_h is the mean structure factor intensity of i observations of symmetry-related reflections with Bragg index h. $R_{cryst}=(\Sigma_h\Sigma_i||F_{obs}|-|F_{calc}||)/\Sigma|F_{obs}|)$ where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes. $R_{free}= \Sigma_{(hkl)\in\tau}||Fobs_{(hkl)}|-k|F_{(hkl)}|/\Sigma_{(hkl)\in\tau}|Fobs_{(hkl)}|$ where the τ set of reflections is omitted from the refinement process. 10% of the data were included in the τ set for calculation of R_{free} and not included in refinement. Values in parenthesis are for the highest resolution shell.

Fig. 2C


A
A′
B′
121
130
140
150
160
170
Apo2L RV TGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLR
TNF-β KP IGD.....PSKQNSLLWRANTDRAFLQDGFSLS
TNF-α KP VAN.....PQAEGQLQWLNRRANALLANGVELR
CD40L QI ISE.....ASSKTTSVLQWAEKGYYTMSNNLVTLE
FasL RK TGK.....SNSRSMPLEWEDTY.GIVLLSGVKYK
RANKL QP TIN.....ATDIPSGSHKVSLSSWYHDR.GWAKISNMTFS
B
C
D
180
190
200
210
Apo2L NG.E HEK YFRFQEEIKENTKNDKQMVQYIYKYTS.YPD
TNF-β NN.S PTS VFSGKAYSPKATSSPLYLAHEVQLFSSQYPF
TNF-α DN.Q PSE LFKGQG....CPSTHVLLTHTISRIAVSYQT
CD40L NGKQ KRQ TFCS.....NREASSQAPFIASLCLKSPG
FasL KG.G NET YFRGQ.....SCNNLPLSHKVYMRNSKYPQ
RANKL NG.K NQD CFRHHETSGDLATEYLQLMVYVTKTSIKIPS
E
F
G
220
230
240
250
260
Apo2L PILLMKSARNSCWSKDAE.....YGLYSIYQ KENDRIFVSVTNE
TNF-β HVPLLSSQKMVYPGLQE.....PWLHSMYH TQGDQLSTHTDGI
TNF-α KVNLLSAIKSPCQRETPEGAEAKPWYEPIYL EKGDRLSAEINRP
CD40L RFERILLRAANTHSSAKP.....CGQQSIHL QPGASVFVNVTDP
FasL DLVMMEGKMMSYCTTGQ.....MWARSSYL TSADHLYVNVSEL
RANKL SHTLMKGGSTKYWSGNSE.....FHFYSINV RSGEEISIEVSNP
H
270
280
Apo2L HLIDMDHE.AS LVG
TNF-β PHLVLSPS.TV AL.
TNF-α DYLLFAESGQV AL.
CD40L SQVSHGTG.FT KL.
FasL SLVNFEES.QT K..
RANKL SLLDPDQD.AT KVR

Figure 3

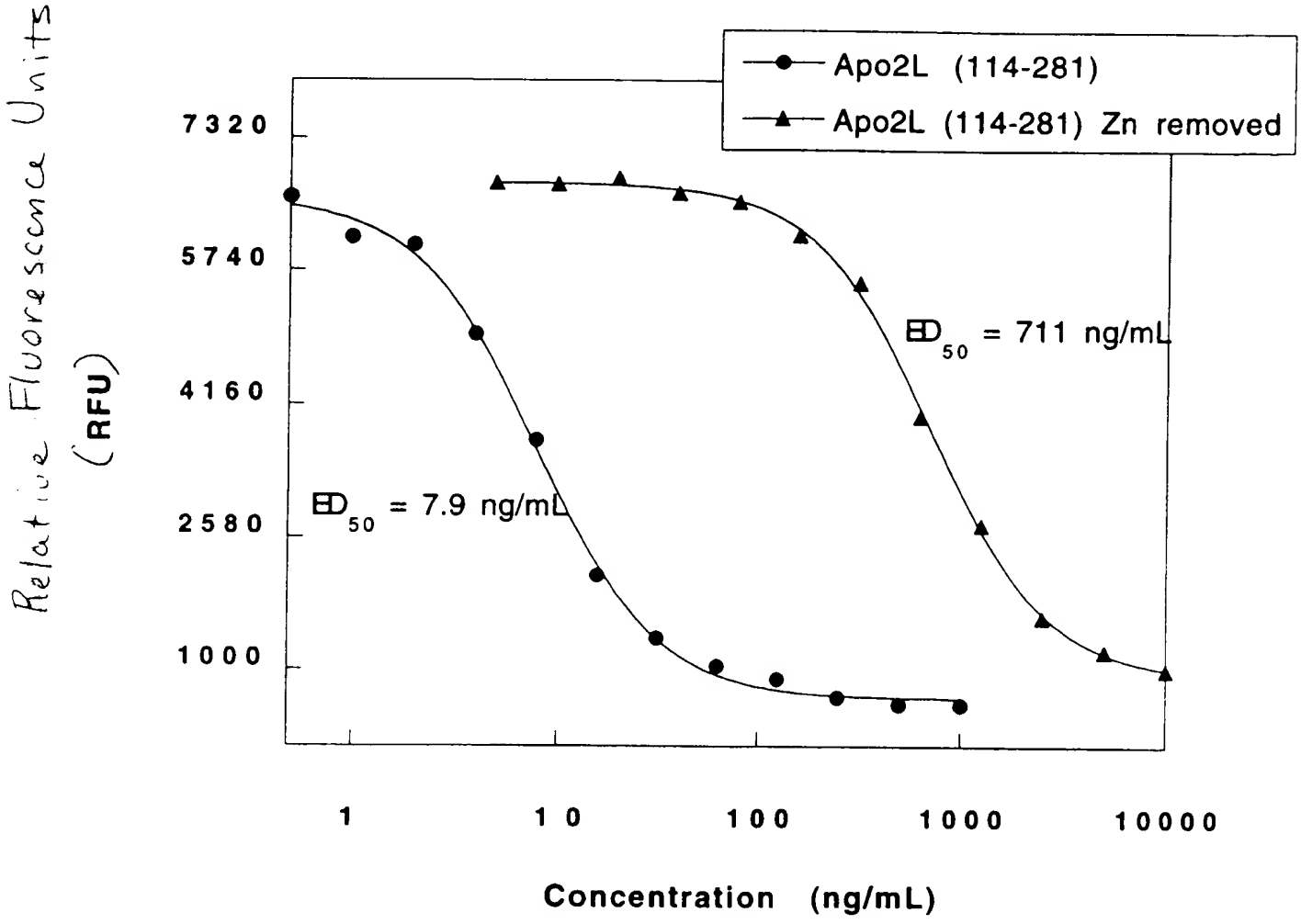

Relative Fluorescence Units (RFU)
7320
5740
4160
2580
1000
Apo2L (114-281)
Apo2L (114-281) Zn removed
ED50 = 711 ng/mL
ED50 = 7.9 ng/mL
1
10
100
1000
10000
Concentration (ng/mL)

Fig. 4

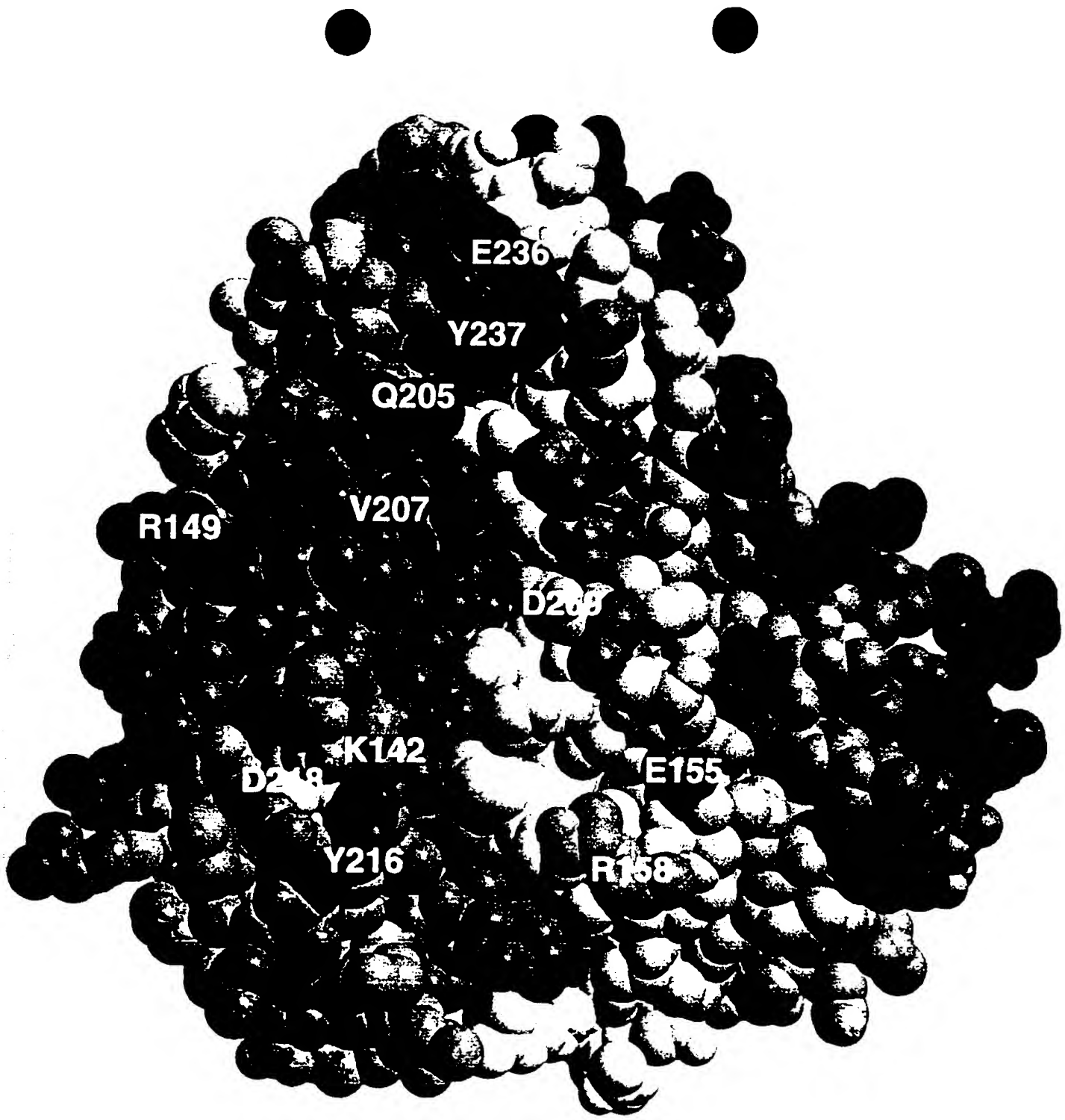


Figure 5

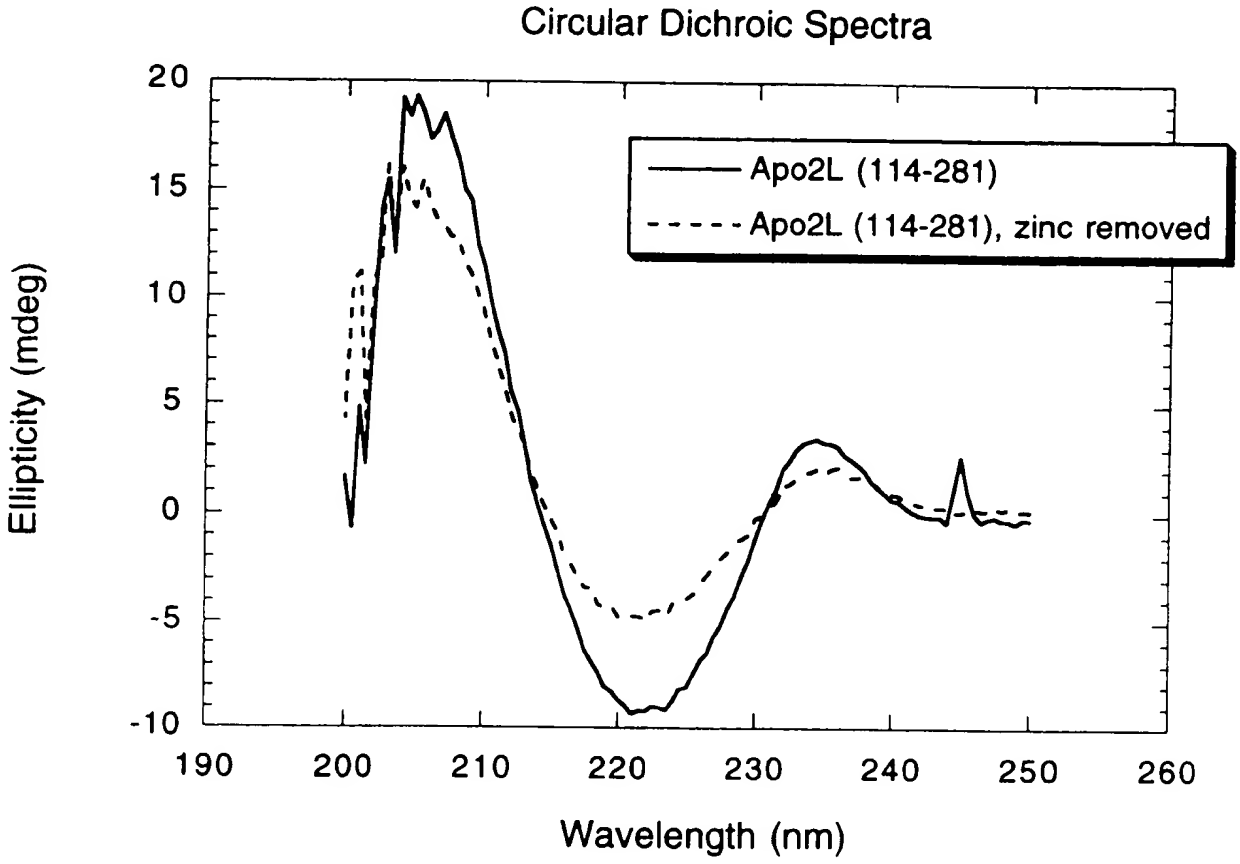

Circular Dichroic Spectra
Apo2L (114-281)
Apo2L (114-281), zinc removed
Ellipticity (mdeg)
20
15
10
5
0
-5
-10
190
200
210
220
230
240
250
260
Wavelength (nm)

Fig. 6

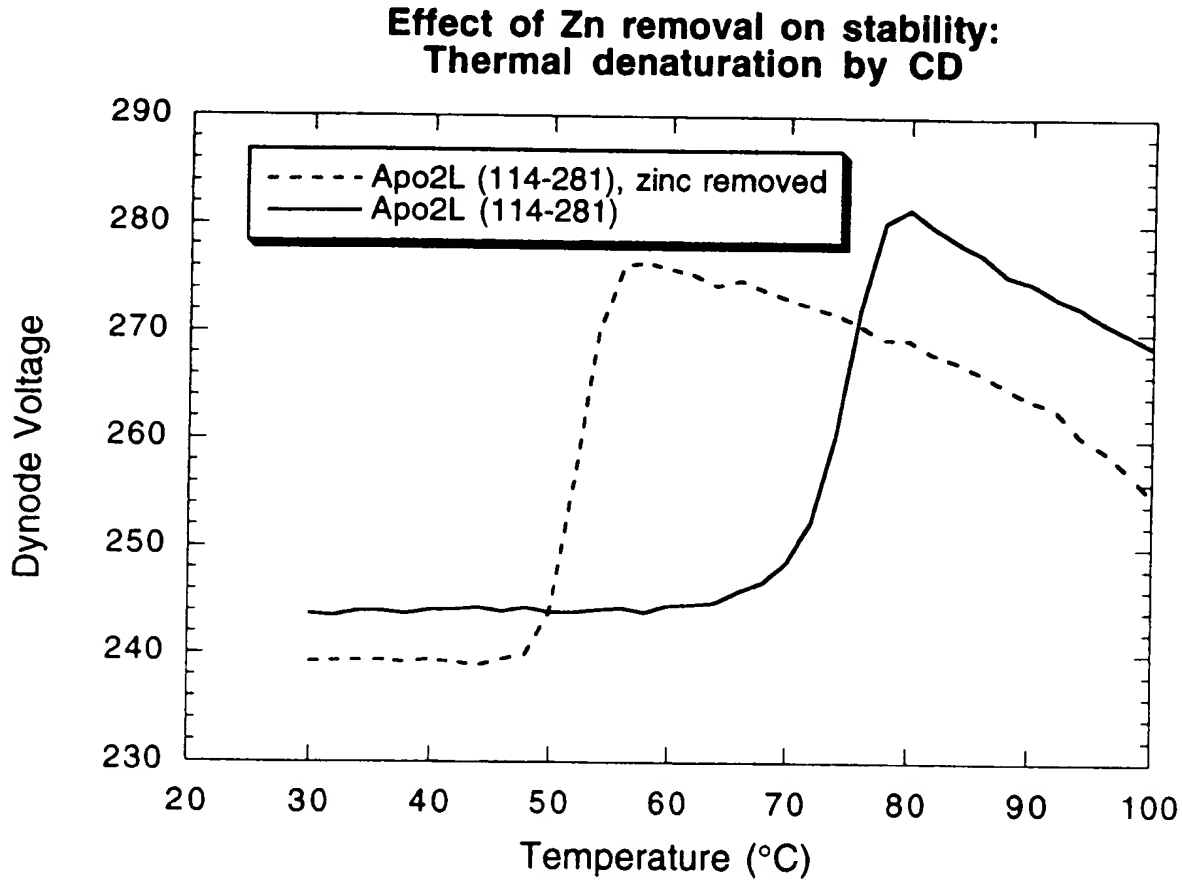

Effect of Zn removal on stability:
Thermal denaturation by CD
Apo2L (114-281), zinc removed
Apo2L (114-281)
Dynode Voltage
290
280
270
260
250
240
230
20
30
40
50
60
70
80
90
100
Temperature (°C)

Fig. 7

Effect of ZnSO4 Additions on Apo2L Product Accumulation

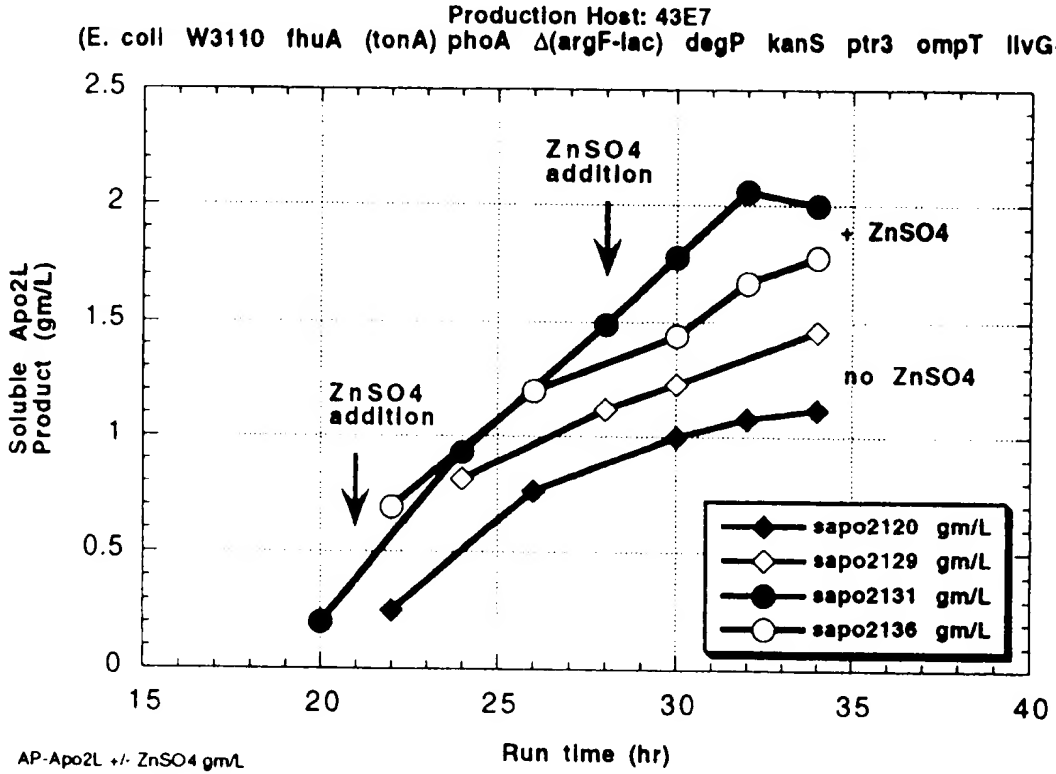
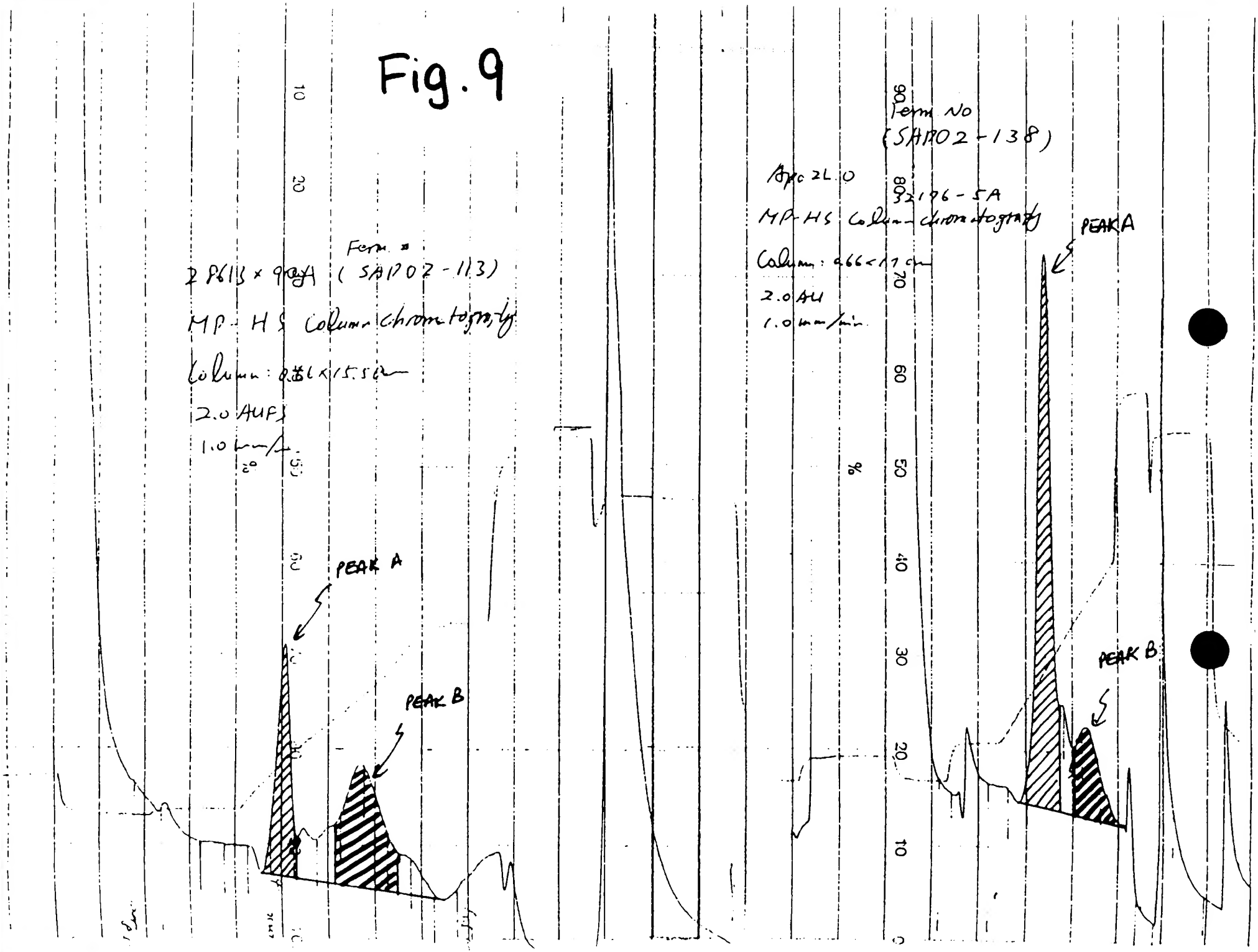


Fig. 8

Fig. 9


Form No
(SA1202-113)
MP-HS Column chromatography
PEAK A
PEAK B
Form No
(SA1202-138)
MP-HS Column chromatography
PEAK A
PEAK B
%

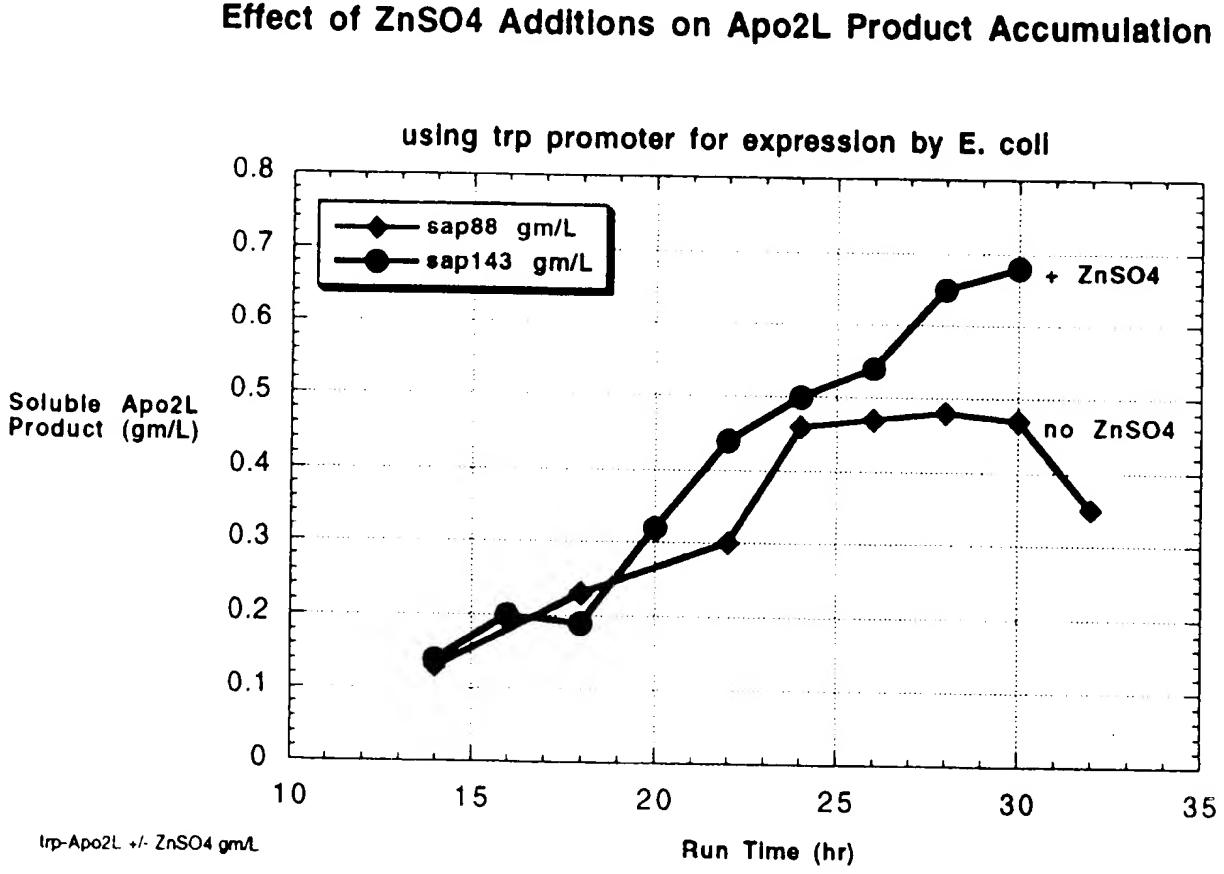

Effect of ZnSO4 Additions on Apo2L Product Accumulation
using trp promoter for expression by E. coli
Soluble Apo2L Product (gm/L)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
sap88 gm/L
sap143 gm/L
+ ZnSO4
no ZnSO4
10
15
20
25
30
35
Run Time (hr)
trp-Apo2L +/- ZnSO4 gm/L

Fig. 10

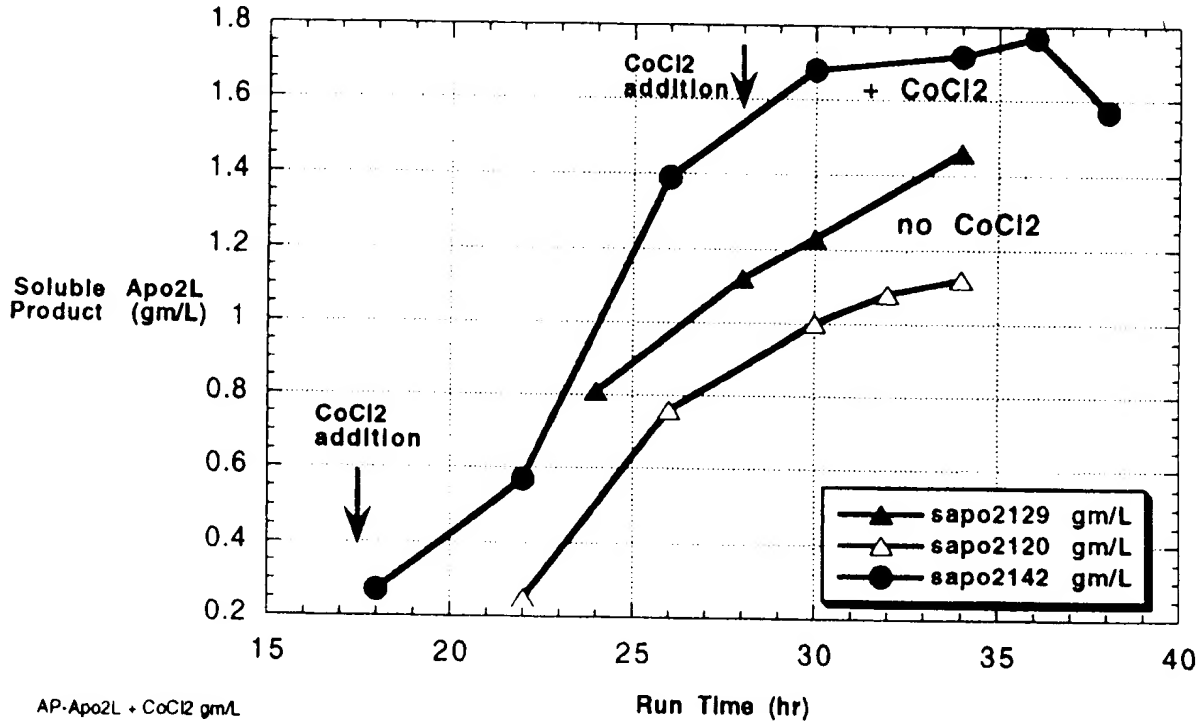

Effect of CoCl2 additions on Apo2L Product Accumulation
Soluble Apo2L Product (gm/L)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
15
20
25
30
35
40
Run Time (hr)
CoCl2 addition
+ CoCl2
no CoCl2
CoCl2 addition
sapo2129 gm/L
sapo2120 gm/L
sapo2142 gm/L
AP-Apo2L + CoCl2 gm/L

Fig. 11

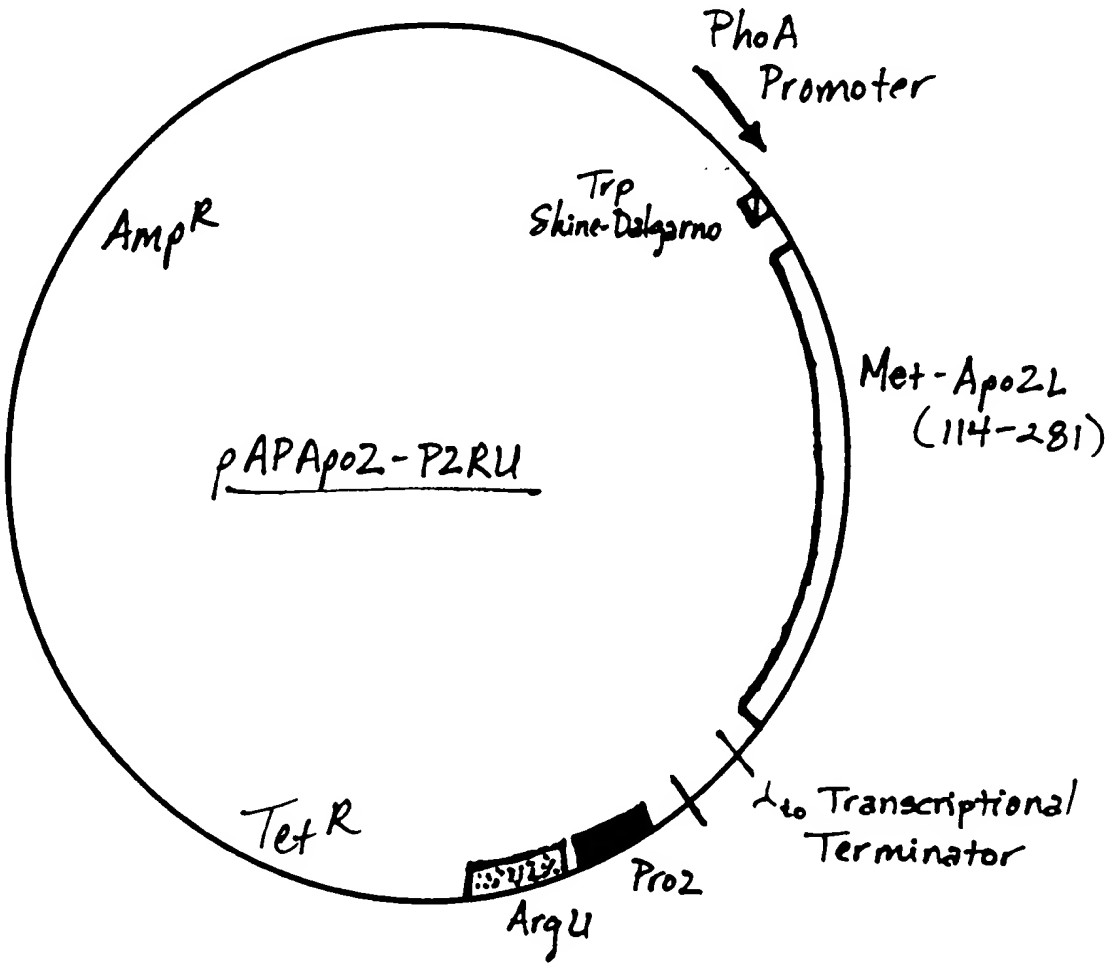

PhoA
Promoter
Trp
Shine-Dalgarno
AmpR
Met-Apo2L
(114-281)
pAPApo2-P2RU
TetR
λto Transcriptional
Terminator
Pro2
ArgU

Fig. 12

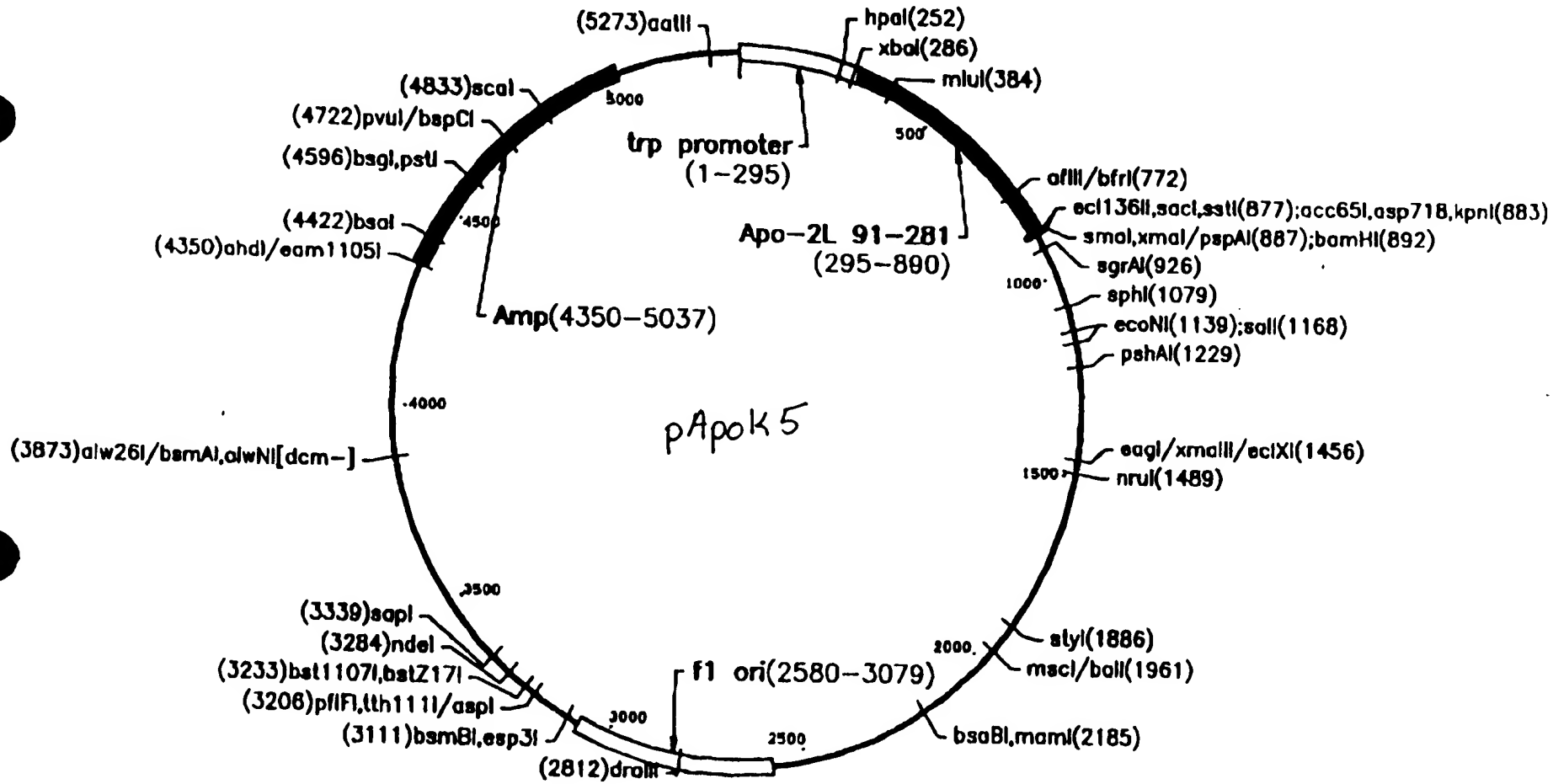

(5273)aatII
hpaI(252)
xbaI(286)
mluI(384)
(4833)scaI
(4722)pvuI/bspCI
(4596)bsgI,pstI
(4422)bsaI
(4350)ahdI/eam1105I
trp promoter
(1-295)
Apo-2L 91-281
(295-890)
Amp(4350-5037)
pApok5
aflII/bfrI(772)
ecl136II,sacI,sstI(877);acc65I,asp718,kpnI(883)
smaI,xmaI/pspAI(887);bamHI(892)
sgrAI(926)
sphI(1079)
ecoNI(1139);salI(1168)
pshAI(1229)
eagI/xmaIII/eclXI(1456)
nruI(1489)
styI(1886)
mscI/ball(1961)
bsaBI,mamI(2185)
f1 ori(2580-3079)
(2812)drdII
(3111)bsmBI,esp3I
(3206)pflFI,tth111I/aspI
(3233)bst1107I,bstZ17I
(3284)ndeI
(3339)sapI
(3873)alw26I/bsmAI,alwNI[dcm-]
500
1000
1500
2000
2500
3000
3500
4000
4500
5000

Fig. 13